Coincident Exposure of Phosphatidylethanolamine and Phosphatidylserine on the Surface of Irradiated Endothelial Cells

 # 1053

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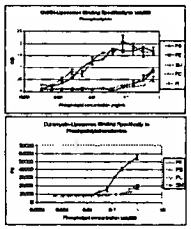
Introduction

Cell membranes are composed of various phospholipids which are non-homogeneously distributed among the inner and outer leaflets. The predominant phospholipids in the outer leaflet are phosphatidylcholine (PC) and sphingomyelin (SM), while phosphatidylserine (PS) and phosphatidylethanolamine (PE) are restricted to the inner leaflet. PS internalization across cell membranes is due to the activity of the enzyme aminophospholipid translocase, which is ATP-dependent and inhibited by calcium. Aminophospholipid translocase transports PE as well as PS. We hypothesized that PE translocation to the outer leaflet of the cell membrane would be coincident with that of PS because the same translocase is responsible of both lipids. To test our hypothesis we induced PE and PS exposure on endothelial cells using irradiation. We than double stained the cells using a peptide (duremycin) which binds specifically to PE and a novel antibody that binds PS and not PE. Irradiation increased the staining intensity for both PS and PE. PS and PE were coincidentally expressed.

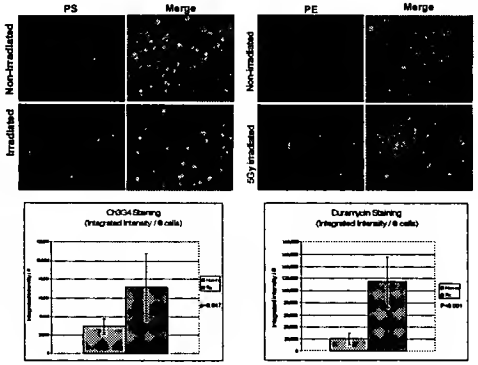
Detection system

We performed fluorescence microscopy to detect PS and PE on the surface of the endothelial cells. For increased sensitivity, we used either liposomes filled with a water soluble fluorophore (pyranine), or multiple biotinylated liposomes. The liposomes were chemically conjugated with ligands that recognize phospholipids. Using a solid phase essay, we showed that liposomes conjugated to the Fab' fragment of an antibody (Ch3G4) detect anionic phospholipids (including PS) and do not bind PE. Liposomes conjugated to the peptide duramycin (derived from *Streptoverticillium cinnamoneus)* specifically recognized PE.



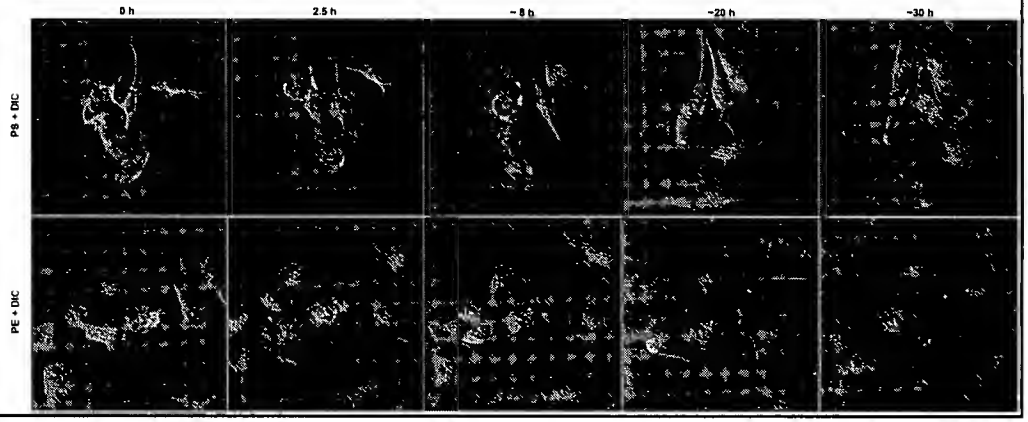
Aim 1 – PS and PE are exposed on the surface of the irradiated endothelial cells

Adult bovine aortic endothelial cells (ABAEs) were irradiated with 5Gy and 24 h later stained with liposomes recognizing PS or PE. Live-cell microscopy was used to obtain fluorescence and phase-contrast images. The staining intensity was quantified as a ratio of integrated intensity of the fluorescence (computed using Image J software) and the number of cells.



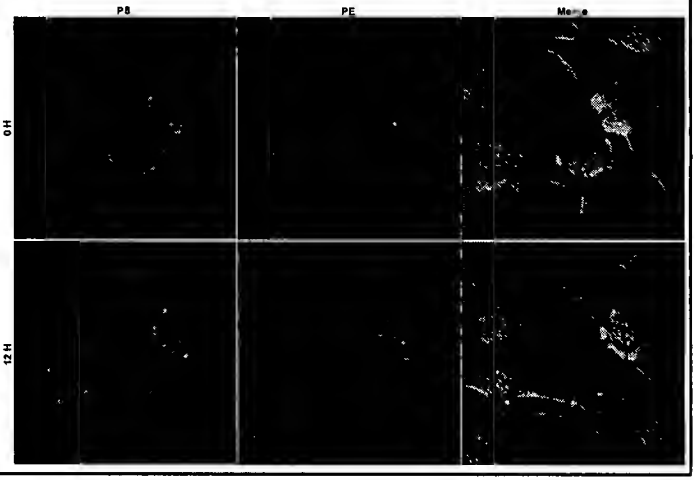
Aim 2 – The dynamics of PS and PE exposure areas are similar

The dynamics of the PS and PE exposure area on the surface of irradiated ABAEs were observed over a 30 h period. The cells were stained with pyrenine-filled liposomes. We followed the time-evolution of the fluorescence. The images presented are obtained by merging fluorescence with DIC pictures. PS and PE exposure is concentrated into blebs or strings of blebs distributed in various locations over the cell surface. In time, the positive areas congregate toward the periphery of the cell.



Aim 3 – PS and PE exposure areas are colocalized on the surface of irradiated endothelial cells and stay together during subsequent movement on the cell surface

Irradiated ABAEs were double stained with pyranine filled liposomes recognizing PS (green) and biotinylated liposomes recognizing PE (detected with streptavidin-Alexa Fluor 594, red). The live cells were pictured at time 0 h and 12 h (without restaining). Positive cells exposed both PE and PS on their surface. The exposure areas for the two markers have high colocalization (yellow). Also, the PE and PS positive areas evolve together in time on the cell surface.



Conclusions

Our results indicate that one of the responses of endothelial cells to irradiation is externalization of phospholipids. PS and PE are externalized together. They appear simultaneously on the same cell surface regions and stay together during subsequent movement on the cell surface. Possibly, inhibition of aminophospholipid translocase occurs after irradiation, leading to coincident exposure of its substrates, PS and PE, on the cell surface

EXHIBIT 9